bio-protocol Preprint

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Dissection of human and mouse embryonic lungs and set-up of organoid culture

Emma L Rawlins

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An abbreviated version of this protocol was published in eLIFE in Jun 2017

Human embryonic lung epithelial tips are multipotent progenitors that can be expanded in vitro as long-term self-renewing organoids

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Detailed protocol

Human fetal lung dissection

- Collect lungs in Hibernate A medium & keep it at 4^{o}C
- On the dissection day need to:
  - Thaw Matrigel in the back of the tissue culture room fridge (takes 3 hr to thaw)
  - Place a 48-well plate (greiner bio-one, Cellstar, Cat No 677102) in the big incubator to warm up the plates, so that the Matrigel will set quickly and form a mound instead of spreading all over the well.
- Wash tissues with T/C PBS.
- Use forceps to remove esophagus and trachea.
- Treat lung with dispase (8 U/ml PBS) at room temperature for 2 min. (Cut lungs into smaller pieces if big.)
- Wash off dispase with PBS & keep the lungs in Advanced+++ DMEM/F12 on ice.
- Dissect a small piece of lung under microscope in a 5 cm petri-dish containing some Advanced+++ DMEM/F12; remove mesenchyme to reveal tips with tungsten needles.
- Cut tips with a sharp tungsten needle on the bud necks.
- Transfer tips with a 50 ul pipette to another petri-dish containing some Advanced+++ DMEM/F12.
- At the same time, prepare cold Matrigel in Eppendorf tubes in a T/C hood.
  - Cool Matrigel and 1.5ml Eppendorf tubes in an ice box.
  - 30ul Matrigel per Eppendorf tube.
- Group 5 tips together and pipette them to a Matrigel Eppendorf prepared above with a 50 ul pipette under microscope. Keep on ice.
- After all the tips have been transferred to the Matrigel eppendorfs, pipette the tips containing Matrigel above to a 48-well plate.
  - Avoid bubbles
  - ~ 25 ul each well
  - ~ 5 tips per well
- Place the 48-well plate in a 37^{o}C incubator for 5 min to solidify the Matrigel
- Add self renewal medium along the side of the well (300 ul per well).
- Fill empty wells with PBS to minimise evaporation from Matrigel wells.
- Incubate in a 37oC CO2 incubator.
- Change medium on Tuesday and Friday; prepare fresh medium weekly
- Passage:
  - 1:4 dilution for the first passage, and then 1:5 dilution for further passages

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Rawlins, E. (2021). Dissection of human and mouse embryonic lungs and set-up of organoid culture. Bio-protocol Preprint. bio-protocol.org/prep1383.
2. Nikolić, M. Z., Caritg, O., Jeng, Q., Johnson, J., Sun, D., Howell, K. J., Brady, J. L., Laresgoiti, U., Allen, G., Butler, R., Zilbauer, M., Giangreco, A. and Rawlins, E. L.(2017). Human embryonic lung epithelial tips are multipotent progenitors that can be expanded in vitro as long-term self-renewing organoids. eLIFE. DOI: 10.7554/eLife.26575

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